

CLAIMS

1. An expression vector which can inducibly express a foreign gene with an inducing substance in a host cell and can be expressed at a temperature equal to or below the suitable growth temperature range of a host other than said host.
2. An expression vector which can inducibly express a foreign gene with an inducing substance in a host cell and can be expressed at a temperature of 15°C or lower.
3. The expression vector according to claim 1 or 2 which can be expressed at 4°C.
4. The expression vector according to any one of claims 1 to 3 wherein the host cell is a bacterium of genus *Rhodococcus*.
5. The expression vector according to claim 4 wherein the bacterium of genus *Rhodococcus* is selected from the group consisting of *R.erythropolis*, *R.fascians* and *R.opacus*.
6. The expression vector according to any one of claims 1 to 5 wherein the inducing substance is thiostrepton.
7. The expression vector according to any one of claims 1 to 6 wherein the foreign gene encodes a protein which inhibits proliferation of the host cell under medium to high temperature conditions exceeding 15°C.
8. The expression vector according to any one of claims 1 to 7 wherein the vector comprises a promoter sequence whose expression can be regulated by an inducing substance and a multicloning site to which a foreign gene can be introduced.
9. A transformant comprising an expression vector according to any one of claims 1 to 8.
10. A method for producing protein using an expression vector according to any one of claims 1 to 8.
11. An inducible expression vector which can inducibly express a gene encoding a protein which inhibits proliferation of the host cell when it is expressed at a temperature within the range of suitable growth temperature of the host cell in another host cell having a suitable growth temperature range lower than the suitable growth temperature of said host cell using an inducing substance.
12. The expression vector according to claim 11 which can be expressed at 4°C.

13. The expression vector according to claim 11 or 12 wherein the host cell is a bacterium of genus *Rhodococcus*.
14. The expression vector according to claim 13 wherein the bacterium of genus *Rhodococcus* is selected from the group consisting of *R.erythropolis*, *R.fascians* and *R.opacus*.
15. The expression vector according to any one of claims 11 to 14 wherein the inducing substance is thiostrepton.
16. The expression vector according to any one of claims 11 to 15 wherein the vector comprises a promoter sequence whose expression can be regulated by an inducing substance and a multicloning site to which a foreign gene can be introduced.
17. A transformant comprising an expression vector according to any one of claims 11 to 16.
18. A method for producing a protein using an expression vector according to any one of claims 11 to 16.
19. An expression vector which can inducibly express a foreign gene using an inducing substance in a bacterium of genus *Rhodococcus*.
20. The expression vector according to claim 19 wherein the bacterium of genus *Rhodococcus* is selected from the group consisting of *R.erythropolis*, *R.fascians* and *R.opacus*.
21. The expression vector according to claim 19 or 20 wherein the inducing substance is thiostrepton.
22. An expression vector according to any one of claims 19 to 21 wherein the vector comprises an expression cassette comprising a *TipA* gene promoter sequence, the first multicloning site to which a foreign gene can be introduced and a transcription termination sequence, an inducer cassette comprising the second promoter sequence and *TipA* gene, a DNA region essential to autonomous replication of a plasmid for a bacterium of genus *Rhodococcus* and a thiostrepton resistance gene.
23. A transformant of a bacterium of genus *Rhodococcus* comprising an expression vector according to any one of claims 19 to 22.
24. A method for producing a protein using an expression vector according to any one of claims 19 to 22.

25. An inducible expression vector for a bacterium of genus *Rhodococcus* which can inducibly express a gene encoding a protein whose expression product inhibits proliferation of the host cell under medium to high temperature conditions exceeding 15°C in a bacterium of genus *Rhodococcus* which can proliferate under low-temperature conditions.
26. An inducible expression vector for a bacterium of genus *Rhodococcus* whose expression can be induced in a bacterium of genus *Rhodococcus* in which a foreign gene can be multiplied under low-temperature conditions, wherein the vector comprises an expression cassette comprising a *TipA* gene promoter sequence, the first multicloning site to which a foreign gene can be introduced and a transcription termination sequence, an inducer cassette comprising the second promoter sequence and *TipA* gene, a DNA region essential to autonomous replication of a plasmid for a bacterium of genus *Rhodococcus* and a thiostrepton resistance gene.
27. The inducible expression vector for a bacterium of genus *Rhodococcus* according to claim 26 wherein the vector further comprises a DNA region essential to autonomous replication of a plasmid for *E. coli* and can be reproduced in *E. coli*.
28. The inducible expression vector for a bacterium of genus *Rhodococcus* according to claim 26 or 27 wherein the *TipA* gene promoter is *TipA-LG10* promoter.
29. The inducible expression vector for a bacterium of genus *Rhodococcus* according to any one of claims 26 to 28 wherein the vector is selected from the group consisting of pTip-NH1 having a nucleotide sequence represented by SEQ ID No. 106, pTip-NH2 having a nucleotide sequence represented by SEQ ID No. 107, pTip-CH1 having a nucleotide sequence represented by SEQ ID No. 108, pTip-CH2 having a nucleotide sequence represented by SEQ ID No. 109, pTip-LNH1 having a nucleotide sequence represented by SEQ ID No. 110, pTip-LNH2 having a nucleotide sequence represented by SEQ ID No. 111, pTip-LCH1 having a nucleotide sequence represented by SEQ ID No. 112, pTip-LCH2 having a nucleotide sequence represented by SEQ ID No. 113, pTip-CH1.1, pTip-CH2.1, pTip-LCH1.1 and pTip-LCH2.1.

30. The inducible expression vector for a bacterium of genus *Rhodococcus* according to any one of claims 25 to 29 wherein the bacterium of genus *Rhodococcus* is selected from the group consisting of *R.erythropolis*, *R.fascians* and *R.opacus*.
31. A transformant of a bacterium of genus *Rhodococcus* comprising an inducible expression vector for a bacterium of genus *Rhodococcus* according to any one of claims 25 to 30.
32. A process for producing a protein whose expression product inhibits proliferation of the host cell under medium to high temperature conditions exceeding 15°C wherein the process comprises introducing an inducible expression vector for a bacterium of genus *Rhodococcus* according to any one of claims 25 to 30 comprising as a foreign gene a gene encoding a protein difficult to be expressed under medium to high temperature conditions exceeding 15°C into a bacterium of genus *Rhodococcus* which can proliferate at low temperature; and culturing the above-mentioned bacteria into which an inducible expression vector for a bacterium of genus *Rhodococcus* is introduced using a culture medium comprising thiostrepton under low-temperature.
33. The process for producing a protein at low temperature according to claim 32 wherein the protein difficult to be expressed under medium to high temperature conditions exceeding 15°C is a protein which inhibits proliferation of the host cell under medium to high temperature conditions exceeding 15°C.
34. The process for producing a protein at low temperature according to claim 32 wherein the protein difficult to be expressed under medium to high temperature conditions exceeding 15°C is a protein which forms an inactive inclusion body in *E. coli* when it is made expressed under medium to high temperature conditions exceeding 15°C.
35. A process for producing a protein derived from psychrotrophic bacteria, heterothermic animals or plants living under a low-temperature environment wherein the process comprises introducing an inducible expression vector for a bacterium of genus *Rhodococcus* according to any one of claims 25 to 30 comprising a gene encoding a protein derived from psychrotrophic bacteria, heterothermic animals or plants living under a low-temperature environment into a bacterium of genus *Rhodococcus* which can proliferate at low temperature; and culturing the

above-mentioned bacteria into which an inducible expression vector for a bacterium of genus *Rhodococcus* is introduced using a culture medium comprising thiostrepton under low-temperature.

36. A process for screening a protein difficult to be expressed under medium to high temperature conditions exceeding 15°C wherein the process comprises introducing an inducible expression vector for a bacterium of genus *Rhodococcus* according to any one of claims 25 to 30 comprising a foreign gene into a bacterium of genus *Rhodococcus* which can proliferate at low temperature; culturing the above-mentioned bacteria into which an inducible expression vector for a bacterium of genus *Rhodococcus* is introduced using a culture medium comprising thiostrepton under medium to high temperature conditions exceeding 15°C and under low-temperature condition; and selecting a gene which can be expressed only under low-temperature condition equal to or below 15°C.

37. The process for screening a protein difficult to be expressed under medium to high temperature conditions exceeding 15°C according to claim 36 wherein the protein difficult to be expressed under medium to high temperature conditions exceeding 15°C is a protein which inhibits proliferation of the host cell under medium to high temperature conditions exceeding 15°C.

38. A process for screening a protein difficult to be expressed under medium to high temperature conditions exceeding 15°C wherein the process comprises selecting a gene which does not express or inhibits proliferation of *E. coli* when it is introduced and made expressed under medium to high temperature conditions exceeding 15°C; and then introducing an inducible expression vector for a bacterium of genus *Rhodococcus* according to any one of claims 25 to 30 comprising said gene as a foreign gene into a bacterium of genus *Rhodococcus* which can proliferate at low temperature; and selecting a gene which can be expressed when cultured under low-temperature condition using a culture medium comprising thiostrepton.

39. The process for screening a protein according to claim 38 wherein the protein difficult to be expressed under medium to high temperature conditions exceeding 15°C is a protein which inhibits proliferation of *E. coli* at 30°C or higher.

40. The process for screening a protein according to claim 38 wherein the protein difficult to be expressed under medium to high temperature conditions exceeding 15°C is a protein which forms an inclusion body when it is made expressed under medium to high temperature conditions exceeding 15°C.

41. The process for screening a protein according to claim 38 wherein the protein difficult to be expressed under medium to high temperature conditions exceeding 15°C is a protein which inhibits proliferation of the host cell under medium to high temperature conditions exceeding 15°C

42. A protein difficult to be expressed under medium to high temperature conditions exceeding 15°C obtained by a screening process according to any one of claims 36 to 41.